(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is derived from a gene in the host microorganism.

Please cancel claims 17-19, 39, and 40.

Remarks

Claims 1-16, 20-32, 35-38, and 41-45 are pending. Claims 5-7, 15, 21, 22, 25, 26, 36, and 38 have been withdrawn as being directed to a non-elected invention. Claims 17-19, 39, and 40 have been canceled. Claims 1, 3, 23, 27, 29, 30, and 35 have been amended. Claims 41-45 have been added. Claims 1, 27, and 30 have been amended to require that the essential gene correspond to an inactivated native gene of the cell. This amendment is supported at least on page 10, lines 6-10, where the use of a copy of a host gene inactivated in the host cell as the essential gene is described. Claims 3, 23, 29, 30, and 35 have all been amended to limit the cell to member of *Enterobacteriaceae*. This amendment is supported at least on page 32, lines 27-29, and page 30, lines 13-16, where use of *Enterobacteriaceae* is described.

Claims 41-45 have been added to more clearly recite what applicants consider to be their invention. New claim 41 requires that the essential gene be a gene essential for metabolism or growth of the cell. New claim 41 is supported at least on page 12, lines 26-28. New claim 42 requires that the essential be a gene essential for cell wall or cell membrane integrity. New claim 42 is supported at least on page 13, line 7. New claim 43 requires that the essential gene be a modification methylase gene, a gene required for nucleic acid replication, or a gene encoding an

enzyme that catalyzes steps in the biosynthesis of DAP. New claim 43 is supported at least on page 13, lines 3-6 and 19-21. New claim 44 requires that the essential gene be an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab*, gene, a *fad* gene, or a *pls* gene. New claim 44 is supported at least on page 13, lines 26-27; page 14, line 12; page 15, lines 6-25; and page 16, lines 1-3.

New claim 45 is similar to, and finds support in, original claim 1. Claim 45 requires that the essential gene be derived from a gene in the host microorganism. This is supported at least on page 12, lines 29-30. A copy of all of the pending claims as they are believed to have been amended is attached to this Amendment and Response in an appendix.

The present invention is a microbial cell having an Environmentally Limited Viability System (ELVS) such that the cell is viable in a permissive environment and non-viable in a non-permissive environment (see page 5, lines 14-19). The ELVS achieves this environmentally specified viability using two components, an essential gene and a lethal gene (see page 5, lines 16-19; claims 1 and 27). Essential genes and lethal genes are specifically limited in the claims, and defined in the specification (see pages 11-17, especially pages 11 and 16), to refer to mutually exclusive genes having mutually exclusive effects. All of the claims now require that the essential gene correspond to an inactivated native gene of the cell (claims 1-16, 20-32, 35-38, and 41-44) or that the essential gene be derived from a gene in the host microorganism (claim 45).

Rejections Under 35 U.S.C. § 102

1. Claims 1-4, 8, 10-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 5,702,916 to Molin et al. Applicants respectfully traverse this rejection.

Molin et al. discloses a cell containment system involving a lethal gene (for example, the *hok* gene) regulated by an environmental condition. Molin et al. also discloses that the cell containment system can include a gene that regulates the expression of the lethal gene (for example, the *sok* gene). Molin et al. fails to disclose or suggest the use of an environmentally regulated essential gene that is expressed under conditions where the lethal gene is not, and not expressed under conditions where the lethal gene is expressed. Molin et al. also fails to disclose an environmentally regulated gene the expression of which is essential to cell viability and which corresponds to an inactivated native gene of the cell.

The present rejection is premised on the identification of the *sok* gene of Molin et al. as an essential gene. While applicants continue to disagree with this characterization and believe that the prior claims are novel over Molin et al.[1], the claims have been amended to exclude genes

[1] Gerdes *et al.* (page 3119, second column, first and second full paragraphs) and Molin *et al.* (column 31, lines 38-65) both disclose use of the temperature regulated *hok* gene and the temperature regulated *sok* gene in the same cell. The *sok* gene is expressed (due to derepression) at 42°C (the permissive temperature). While the *hok* gene is transcribed (due to derepression) at 42°C, the *hok* transcript is not translated at 42°C since the *sok* transcript prevents its translation. However, neither the *hok* gene nor the *sok* gene are expressed at 30°C (the non-permissive temperature). Expression of both the *hok* gene and the *sok* gene are repressed by the CI857 repressor at the lower temperature. Thus, the expression pattern disclosed by Gerdes *et al.* and Molin *et al.* can be characterized as follows:

such as the *sok* gene for use as an essential gene. Specifically, the claims have been amended to require that the essential gene correspond to an inactivated native gene of the cell. The *sok* gene disclosed in Molin et al. is carried on a vector introduced into the cells of Molin et al. The *sok* gene does not correspond to any inactivated native gene of the cell (nor any native gene of the cell, active or inactive). Thus, Molin et al. fails to disclose each and every feature of the claimed cells. Accordingly, Molin et al. fails to anticipate the claimed cells and method.

Table 1
The Cited Publications

	Permissive environment 42°C	Non-permissive environment 30°C
hok gene (lethal gene)	Not expressed	**Not expressed**
sok gene ("essential" gene)	Expressed	Not expressed

The present claims require that the essential gene be expressed when the cell is in the permissive environment but not expressed when the cell is in the non-permissive environment. The present claims also require that the lethal gene be expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment. Taking the *hok* gene as a lethal gene and, in arguendo, assuming the *sok* gene to be an essential gene, the claims require the following expression pattern:

Table 2
The Claimed Cells

	Permissive environment 42°C	Non-permissive environment 30°C
hok gene (lethal gene)	Not expressed	**Expressed**
sok gene ("essential" gene)	Expressed	Not expressed

As can be seen, the expression pattern of the cells disclosed by Gerdes *et al.* and Molin *et al.* (Table 1) is not the same as the expression pattern required by the claims (Table 2). Thus, neither Gerdes *et al.* nor Molin *et al.* disclose each and every feature of the claimed cells. Accordingly, neither Gerdes *et al.* nor Molin *et al.* anticipate the claimed cells and method.

2. Claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120 (1986). Applicants respectfully traverse this rejection.

Gerdes *et al.* (PNAS) disclose (page 3119, second column, and Fig. 3) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene as defined in the specification. Gerdes *et al.* (PNAS) also separately discloses a *sok* gene regulated by the temperature-sensitive λCI_{857} repressor. The *sok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *sok* gene regulates expression of the *hok* gene. Thus, the *sok* gene can be considered a regulatory gene as defined in the specification. Gerdes *et al.* (PNAS) fail to disclose any environmentally regulated *essential* gene. Gerdes et al. also fails to disclose an environmentally regulated gene the expression of which is essential to cell viability and which corresponds to an inactivated native gene of the cell.

The present rejection is premised on the identification of the *sok* gene of Gerdes et al. as an essential gene. While applicants continue to disagree with this characterization and believe that the prior claims are novel over Gerdes et al. (see footnote 1), the claims have been amended to exclude genes such as the *sok* gene for use as an essential gene. Specifically, the claims have been amended to require that the essential gene correspond to an inactivated native gene of the cell. The *sok* gene disclosed in Gerdes et al. is carried on a vector introduced in to the cells of

Gerdes et al. The *sok* gene does not correspond to any inactivated native gene of the cell (nor any native gene of the cell, active or inactive). Thus, Gerdes et al. fails to disclose each and every feature of the claimed cells. Accordingly, Gerdes et al. fails to anticipate the claimed cells and method.

Rejections Under 35 U.S.C. § 112, first paragraph

1. Claims 3, 23, 24, 29-32, and 35 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabling only for the use of some *Salmonella* strains as a vaccine. Applicants respectfully traverse this rejection.

A. Applicants initially note that the rejection is based on a misinterpretation of the claimed cells. The claimed Environmentally Limited Viability System (i.e. the regulated essential and lethal genes recited in the claims) is **not** a bacterial attenuation system. Rather, the environmentally regulated expression of the lethal and essential genes results in cell **viability** in the permissive environment and cell **non-viability** in the non-permissive environment. Attenuation of virulent bacteria is completely different and represents a feature that can be **combined** with the claimed Environmentally Limited Viability System. Where appropriate, the claimed Environmentally Limited Viability System can be embodied in a host cell that has been attenuated. Such attenuation is well known and is thoroughly described in the specification.

Applicants also note that the claimed cells need not produce a robust immune response (although many of the claimed cells will have this ability). Only claims 23-26 and 30-35 recite the use of the cells as a vaccine or a method of inducing immunoprotection. The specification (page 39) defines vaccine as an agent used to stimulate the immune system of a living organism

so that an immune response occurs. Significantly, the specification states that *preferred* vaccines are sufficient to stimulate the immune system of a living organism so that protection against future harm is provided, indicating that the vaccine need not induce immunoprotection. Only claims 30-35 require this level of immune response. While the rejection implies that some mutations used in the Environmentally Limited Viability System (that is, *asd* and *pur* mutations) will render the claimed cells incapable of producing immunoprotection. However, as described in the specification, mutations in essential genes such as *asd* and *pur* are *complemented* by an environmentally regulated form of the essential gene. Thus, the claimed cells are not, in fact, Asd- or Pur- (when *asd* or *pur* is used as the essential gene) since the environmentally regulated versions of the genes provide the necessary function. Thus, even if an *asd* or *pur* mutant *Salmonella* strain exhibited reduced immunogenicity (as asserted in the rejection), such a result is not relevant to the claimed cells since the claimed cells remain Asd+ or Pur+ (in the permissive environment).

B. Notwithstanding the above, applicants have amended claims 3, 23, 24, 29-32, and 35 to be limited to cells that belong to the *Enterobacteriaceae*. This is the group of bacteria to which *Salmonella* and *E. coli* belong. Extensive guidance is provided in the specification for the use of *Enterobacteriaceae* as host cells. Numerous specific examples of the claimed environmentally limited viability system using *Salmonella* and reference to the ready extension of such examples in other enteric bacteria provide further support and guidance for practice of the claimed system in analogous *Enterobacteriaceae*. *Enterobacteriaceae* are the most studied and manipulated organism with numerous techniques and procedures developed for the growth,

genetic alteration, and use of such bacteria. Given the guidance provided, the knowledge in the art, and the analogous genetic makeup of *Salmonella* and other *Enterobacteriaceae*, applicants submit that it would not require undue experimentation to practice the claimed environmentally limited viability system across the full scope of host cells as now claimed.

2. Claims 39 and 40 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which is not described in the specification in such a way as to convey that applicants possessed the claimed subject matter at the time of filing. Applicants respectfully traverse this rejection.

While applicants assert that the subject matter of claims 39 and 40 is sufficiently described in the specification[2], claims 39 and 40 have been cancelled in order to facilitate

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[2] The standard regarding what is or is not supported by the specification has been clearly articulated as requiring the specification to convey with reasonable clarity to those skilled in the art that, as of the filing date sought, the inventor was in possession of the invention, i.e., whatever is now claimed. *Vas-Cath, Inc. v. Mahurkar,* 935 F.2d 1555 19 USPQ2d 1111, 1117 (Fed . Cir. 1991). In this regard, applicants also direct attention to MPEP § 2163.02 which describes the standard to be applied in determining if the written description requirement is satisfied. MPEP § 2163.02 reads, in pertinent part:

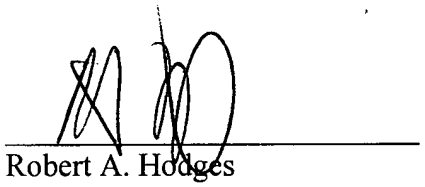
> Whenever the issue [of adequacy of the written description] arises, the fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed. The subject matter of the claim need not be described literally (i.e., using the same terms or in haec verba) in order for the disclosure to satisfy the description requirement. (emphasis added)

Applicants note that trans regulatory elements are defined as a molecule or complex that modulates the expression of a gene, and that trans regulatory elements are described in a section of the specification devoted to elements for regulation of the lethal and essential genes described elsewhere in the specification. The specification makes clear that regulatory elements in the claimed Environmentally Regulated Viability System are separate from the essential and lethal genes. The examples all involve the use of regulatory elements that are separate from the lethal and essential genes that are the object of regulation. In particular, essential genes are never used in the specification to regulate expression of a lethal gene. Thus, applicants assert that the concepts recited in claims 39 and 40 are clearly conveyed in the specification. This is all that is required.

prosecution of the application. With the cancellation of claims 39 and 40, applicants submit that the present rejection is moot.

Allowance of claims 1-16, 20-32, 35-38, and 41-45 is respectfully solicited.

Respectfully submitted,



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Chandra Russell

Chandra Russell

Date: September 14, 1999

Appendix: Claims As Pending After Amendment

1. (Amended) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene corresponds to an inactivated native gene of the cell.

2. (Unamended) The cell of claim 1 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

3. (Amended) The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,

wherein the cell is a member of *Enterobacteriaceae*.

4. (Unamended) The cell of claim 1 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

5. (Unamended) The cell of claim 4 wherein the lethal gene is carried on an extrachromosomal vector and expression of the lethal gene is regulated by an expression product of a regulatory gene.

6. (Unamended) The cell of claim 5 wherein the expression product of the regulatory gene inhibits expression of the lethal gene and is expressed or active only in the permissive environment.

7. (Unamended) The cell of claim 5 wherein the expression product of the regulatory gene induces expression of the lethal gene and is expressed or active only in the non-permissive environment.

8. (Unamended) The cell of claim 4 wherein the vector has two lethal genes.

9. (Unamended) The cell of claim 8 wherein the vector comprises pMEG-104.

10. (Unamended) The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. (Unamended) The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. (Unamended) The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. (Unamended) The cell of claim 1 wherein expression of the essential gene is regulated by an expression product of a regulatory gene.

14. (Unamended) The cell of claim 13 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

15. (Unamended) The cell of claim 13 wherein the expression product of the regulatory gene induces expression of the essential gene and is expressed or active only in the permissive environment.

16. (Unamended) The cell of claim 4 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

20. (Unamended) The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

21. (Unamended) The cell of claim 20 wherein the desired expression product is an antigen.

22. (Unamended) The cell of claim 21 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

23. (Amended) The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when

the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C,

wherein the cell is a member of *Enterobacteriaceae*.

24. (Unamended) The cell of claim 23 further comprising an expression gene wherein the expression gene encodes a desired expression product.

25. (Unamended) The cell of claim 24 wherein the desired expression product is an antigen.

26. (Unamended) The cell of claim 25 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

27. (Amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment,

wherein the essential gene corresponds to an inactivated native gene of the cell.

28. (Unamended) The method of claim 27 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

29. (Amended) The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,

wherein the cell is a member of *Enterobacteriaceae*.

30. (Twice amended) A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,

wherein the essential gene corresponds to an inactivated native gene of the cell,

wherein the cell is a member of *Enterobacteriaceae*.

31. (Unamended) The method of claim 30 wherein the system further comprising an expression gene wherein the expression gene encodes an antigen.

32. (Unamended) The method of claim 31 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

35. (Amended) The method of claim 30 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of *Enterobacteriaceae*.

36. (Unamended) The cell of claim 5 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the lethal gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

37. (Unamended) The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

38. (Unamended) The cell of claim 17 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

41. (New) The cell of claim 1 wherein the essential gene is a gene essential for metabolism or growth of the cell.

42. (New) The cell of claim 1 wherein the essential is a gene essential for cell wall or cell membrane integrity.

43. (New) The cell of claim 1 wherein the essential gene is a modification methylase gene, a gene required for nucleic acid replication, or a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP.

44. (New) The method of claim 1 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab*, gene, a *fad* gene, or a *pls* gene.

45. (New) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is derived from a gene in the host microorganism.